

Claims:

1. A method for development of nucleotide probes for myctophid fishes, said method comprising the steps of :
 - (i) extracting the DNA from the muscle tissue of a myctophid fish,
 - (ii) selecting gene regions in the extracted DNA with the selected primers and amplifying the same using polymerase chain reaction (PCR),
 - (iii) eluting the PCR amplified DNA,
 - (iv) reamplifying the gene regions from PCR amplified DNA and eluting the same,
 - (v) cycle sequencing of eluted DNA using a single primer,
 - (vi) purifying extension products,
 - (vii) sequencing the extension product on acrylamide gel,
 - (viii) confirming the sequences for the target gene by Blast -Email,
 - (ix) ligating the eluted PCR products in a vector,
 - (x) preparing the electro-competent cells for electro transformation,
 - (xi) electro transforming the host cells,
 - (xii) growing and harvesting of transformed host cells,
 - (xiii) confirming that the transformed bacteria has the plasmids with the gene inserts by PCR.
 - (xiv) purifying recombinant plasmid DNA having the cloned gene probes from the transformed host cells,
 - (xv) checking purity and specificity of the cloned DNA probe insert by cutting with restriction enzyme,
 - (xvi) confirming the molecular size of the DNA probe insert,
 - (xvii) PCR amplification of the gene insert from the probe using both primers,
 - (xviii) eluting of the amplified gene region,
 - (xix) cycle sequencing of the gene region of the probe,
 - (xx) sequencing of the cloned DNA insert on acrylamide gel,
 - (xxi) comparing the DNA sequence of the prepared DNA probes using "BLAST" program "against the known sequences of similar genes in the genome data bases,

- (xxiii) confirming the sequences of the cloned probe by aligning with sequences of the claim 1(vii), and
- (xxiv) designing species specific primers from the sequences.
2. A method as claimed in claim 1 wherein the myctophid fishes are selected from the group comprising *Stenobrachis leucopsarus*, *Diaphus theta*, *Protomyctophum crockeri*, *Tarletonbeania crenularis* and *Lampanyctus regalis*.
3. A method as claimed in claim 1 wherein the gene regions are selected from mitochondrial and nuclear genes.
4. A method claimed in claim 1 wherein the mitochondrial genes taken for probe preparation are selected from the group comprising: Cyt b and D-loop genes, 12 S RNA and 16 S RNA genes.
5. A method claimed in claim 1 wherein the nuclear genes taken for probe preparation are selected from Rod and ITS-2 genes.
6. A method of claim 1 wherein the PCR amplified cleaned nuclear gene probe is Rod gene.
7. A method claimed in claim 1 wherein the nuclear gene taken for the cloned probe preparation is ITS-2 gene.
8. A method as claimed in claim 1 wherein the concentration of primers used for PCR amplification is 20 meu. L.
9. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for amplification and detection of Cyt b gene contains oligonucleotides with the sequences:
- CYT 1: 5' TGA YTT GAA RAA CCA YCG TTG 3'
CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3'
10. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for reamplification and detection of Cyt b gene contains oligonucleotides with the sequences:
CBI-L: 5' CCA TCC AAC ATC TCA GCA TGA TGA AA 3'
CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3'

11. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification and detection of D-Loop gene contains oligonucleotides with the sequences :
PRO-L : 5' CTA CC 3'
D-LOOP H: 5' CCT GAA GTA GGA ACC AGA TG 3'
 12. A method as claimed in claim 1 wherein the forward and backward primers used for PCR amplification of ITS2 gene were
ITS1 F : 5' TTG TAC ACA CCGCCCGTC GC 3'
ITS2 R : 5' ATA TGC TTA AAT TCA GCG GG 3'
 13. A method as claimed in claim 1 wherein the forward and backward primers used for PCR reamplification of ITS2 gene from ITS1 F and ITS2 R PCR amplification were
ITS2 F: 5' CTA CGC CTG TCT GAG TGT C 3'
ITS2 R: 5' ATA TGC TTA AAT TCA GCG GG 3'
 14. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of Rhodopsin gene Rod contains oligonucleotides with the sequences:
ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'
ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'
 15. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 12S RNA gene contains oligonucleotides with the sequences:
12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'
12 SB-H : 5 ' AGA GTG ACG GGC GGT GTG T 3'
 16. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 16S RNA gene contains oligonucleotides with the sequences:
16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3'
16 SBR-H : 5 ' CCG GTC TGA ACT CAG ATC ACG T 3'
 17. A method as claimed in claim 1 wherein the forward and backward primers used for PCR amplification of Rhodopsin gene Rod were :

- ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'
ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'
18. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 12S RNA gene were :
12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'
12 SB-H: 5' AGA GTG ACG GGC GGT GTG T 3'
19. A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 16S RNA gene were :
16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3'
SBR-H: 5' CCG GTC TGA ACT CAG ATC ACG T 3'
20. A method claimed in claim 1 wherein the 12S RNA gene and 16S RNA gene in the myctophid fish *Stenobrachius leucopsarus* were amplified by PCR.
21. A method claimed in claim 1 wherein the 12S RNA and 16S RNA gene in myctophid fish *Diaphus theta* were eluted by PCR amplification.
22. A method claimed in claim 1 wherein the elution of PCR amplification products of myctophid fish *Protomyctophum crockeri*, resulted in 12 S RNA.
23. A method claimed in claim 1 wherein the elution of PCR amplification products of myctophid fish *Protomyctophum crockeri*, resulted in 16 S RNA.
24. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Tarletonbeania crenularis*, resulted in 12 S RNA.
25. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Tarletonbeania crenularis*, resulted in 16 S RNA.
26. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Lampanyctus regalis*, resulted in 12 S RNA.
27. A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Lampanyctus regalis*, resulted in 16 S RNA.
28. A method claimed in claim 1 wherein the cycle sequencing primer concentration used was 2 μ L,
29. A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:
CYT I: 5' TGA YTT GAA RAA CCA YCG TTG 3'

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30. A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:
CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3'
31. A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:
CBI-L: 5' CCA TCC AAC ATC TCA GCA TGA TGA AA 3'
32. A method claimed in claim 1 wherein the cycle sequencing forward primer for D-Loop region consisted of oligonucleotides with the sequence:
PRO-L : 5' CTA CC 3'
33. A method claimed in claim 1 wherein the backward cycle sequencing primer for D-Loop region consisted of oligonucleotides with the sequence:
D-LOOP H: 5' CCT GAA GTA GGA ACC AGA TG 3'
34. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of ITS2 gene consisted of oligonucleotides with the sequence:
ITS 1 -F : 5' TTG TAC ACA CCG CCC GTC GC 3'
35. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of ITS2 gene consisted of oligonucleotides with the sequence:
ITS2 -R : 5' ATA TGC TTA AAT TCA GCG GG 3'
36. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of Rhodopsin gene Rod consisted of oligonucleotides with the sequence:
ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'
37. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing consisted of oligonucleotides with the sequence:
ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'
38. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of 12S RNA gene consisted of oligonucleotides with the sequence:
12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'
39. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of 12S RNA gene consisted of oligonucleotides with the sequence:
12 SB-H : 5' AGA GTG ACG GGC GGT GTG T 3'

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40. A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of 16S RNA gene consisted of oligonucleotides with the sequence:
16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3'
41. A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of 16S RNA gene consisted of oligonucleotides with the sequence:
16 SBR-H: 5' CCG GTC TGA ACT CAG ATC ACG T 3'
42. A method as claimed in claim 1 wherein the extension products of 12 S RNA gene region are purified by conventional methods.
43. A method as claimed in claim 1 wherein the extension products of 16 S gene region are purified by conventional method.
44. A method as claimed in claim 1 wherein the extension products of CYT b gene are purified by conventional method.
45. A method as claimed in claim 1 wherein the extension products of ROD gene are purified by conventional method.
46. A method as claimed in claim 1 wherein the extension products of D-Loop control region are purified by conventional method.
47. A method as claimed in claim 1 wherein the extension products of ITS2 region are purified by conventional method.
48. A method as claimed in claim 1 wherein the extension products of 12 S RNA gene region was sequenced in an automated sequencer.
49. A method as claimed in claim 1 wherein the extension products of 16 S gene region was sequenced in an automated sequencer.
50. A method as claimed in claim 1 wherein the extension products of CYT b gene was sequenced in an automated sequencer.
51. A method as claimed in claim 1 wherein the extension products of ROD gene was sequenced in an automated sequencer.
52. A method as claimed in claim 1 wherein the extension products of D-Loop control region was sequenced in an automated sequencer.
53. A method as claimed in claim 1 wherein the extension products of ITS2 region was sequenced in an automated sequencer.

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54. A method as claimed in claim 1 wherein the identity of the gene 12S RNA is confirmed by Blast Email.
 55. A method as claimed in claim 1 wherein the identity of the gene 16S RNA is confirmed by Blast Email.
 56. A method as claimed in claim 1 wherein the identity of the gene CYT b is confirmed by Blast Email.
 57. A method as claimed in claim 1 wherein the identity of the gene ROD is confirmed by Blast Email.
 58. A method as claimed in claim 1 wherein the identity of the D-Loop is confirmed by Blast Email.
 59. A method as claimed in claim 1 wherein the identity of the gene ITS2 is confirmed by Blast Email.
 60. A method as claimed in claim 1 wherein the vector used for cloning was Bluescript KS' phagemid.
 61. A method as claimed in claim 1 wherein the vector used for cloning had ampicillin resistance gene for selection.
 62. A method as claimed in claim 1 wherein the vector used for cloning had Lac Z gene for blue white colony selection.
 63. A method as claimed in claim 1 wherein the CoIE 1 was the origin for replication of phagemid in the absence of helper phage.
 64. A method as claimed in claim 1 wherein F 1 (-) origin for recovery of antisense strand of lac Z gene when a host strain containing the bluescript II phagemid.
 65. A method as claimed in claim 1 wherein the host cells used for transformation were E. coli blue bacteria (Bacteria Strain XL 1 blue) XL1-Blue :- F' ::Tn10,pro A^rB^rlacI^q (lacZ)M15/recA1endA1gyrA96(Nal^r)thi hsdR17(r_k^rm_k^r)supE44relA1 lac.
 66. A method as claimed in claim 1 wherein probes are containing oligonucleotide sequences are cloned Cyt b , D-Loop, ITS2 and Rod genes.
 67. A method as claimed in claim 1 wherein the probes of CYT b gene is an oligonucleotide sequence named as PSL CYTL.

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68. A method as claimed in claim 1 wherein the probes of ITS 2 gene is an oligonucleotide sequence named as PSL ITS 2F.
69. A method as claimed in claim 1 wherein the probes of D-Loop control region gene is an oligonucleotide sequence named as PSL PROL.
70. A method as claimed in claim 1 wherein the PCR amplified sequence of ROD gene probe is named as ROD SLMB.
71. A method as claimed in claim 1 wherein the PCR amplified sequence of D-Loop gene probe is named as D-Loop SLMB.
72. A method as claimed in claim 1 wherein the PCR amplified sequence of ITS 2 gene probe is named as ITS 2 SLMB.
73. A method as claimed in claim 1 wherein the PCR amplified sequence of Cyt b gene probe is named as Cyt L SLMB.
74. The nucleotide base sequences of PSL CYTL (748 bp) comprising :

5'

CTTNCCCAATT	TTGGGCGCTT	NGGCNCGCTN	CTCCNCGAGA	CTCTCGGTAN
TAATCCAANT	CNCTNCGGGC	CNCTCCCTAC	CANTNCNCTA	CACCNCAAAT
TNCAACCCNG	TTTCCTCATC	ANTCAACCAC	ATCTGTCGAA	AACNTCAACT
ACGGGCTGACT	AATCCGAAAA	CATGCACGCT	AACCGTGCCCT	CTTTCTTCTT
CATCTGTATT	TATCTNCNCN	TTGGANGAGG	ACTATNCTAC	GGATCCTTAC
TCTACGAAGA	GACGTGAGGT	GTGGGTGTTA	TTCTTCTCCT	TCTAAATAATG
ATGACTGCNT	TTGTTGGCTA	TGTGCTNCCC	NGAGGACAAA	TGTCCTTITG
AGGTGCTACT	GTCATTACAA	NCCTACTCTC	TGCTGTNCCG	TNTGTTNGCG
GCNCTCTANT	TCAATGAATT	TGAGGTGGCT	TCTCCGTAAA	CACGCAACGC
TCACTCGTTT	CTTCGCGNTTC	CACTTCTTGT	TCCCATTGTT	TGTCGNGCT
ATAACCNNGG	TTCACCGNGAT	TNNCCGACAT	CAAACAGGCT	CTAAANCCCC
CCCGGNTTGA	CTCCATACAA	CAAACACCTC	CACCCATTTC	NCTATAAAAC
TCTAGGTTCG	TGCCCGTATT	GGCTTACTTC	ATGNCTATT	CCCNNGCGA
GGGACNAAAA	TTCTGCACC	CCCTCCCCNC	AAAATAAANA	ATGTGCTNT
CCTACCANAA	AACAAACNNAN	ACGGGGTNTG	CNCTTCCATC	ATCCACN 3'

75. The nucleotide base sequences of PSLITS2F comprises :(225BP)

5'			
TCTACGATCT	ACCGGCNTTT	NNTGTGGAAA	GACGATCATG
CATTTATGTG	TGTCTTTCTA	TGGATTGAA	CCGTGTGGTA
CGTCTTGCG	TACTGCTTGG	AAGGCTAAC	TTGCTTCTGT
CCTTCCTTGTG	CAGTCTCGCA	CTGTCTATGC	AACGTGTCT
ACTTCGACTT	CTGTGAAAAA	ATCTTACTTT	TGACCTCAGA
TCAGACAAGA	CTACCCGCTG	AATTT	3'

76. The nucleotide base sequences of PSL PROL comprises :(749 BP)

5'			
CCTTTCCGN	ATAGGCCCAN	CTCAAATGAA	TTCCCTCTCT
CCTGGTCCAA	GCCCAAATG	TGGACGGCAG	TTGACAATG
GTTACAAATC	GTGACAATC	GGCTACATAA	TTGCCGATAG
CGATGTCGT	AAACCAAGTC	AAACAAATGCG	CGATGTATAT
CGGCCAAACC	CATATATGGG	TCTGGCTGTA	TTITGTGTTG
AGCAACGTCA	CACCACTGTC	TGGTCAGCAT	ATAAGATGTT
GACATCTTGC	AACATCTTAC	CCACAGACAG	ACAGTACCG
CTGCTTACGA	ANGGGCTAG	TGTTGTTGTG	AGAACGAG
ATACATACGT	CAAACAGACG	CCGGTGCACT	TGAAGACACT
GTTGAAGGT	GCCGCACTAC	TTGACAGACA	GCCCATGATG
CGCTGGACAG	TGACCAAAGC	TACNGGAGGA	CCANATGGA
ATCCGTGTTG	CGTTGCCGTG	GGACTCAAGT	TGTACACTTT
TGGATGGTTG	ATCACTANAN	CCGCTGCCGG	GAGAACACT
CGCTCCTGTT	TCACTAATCA	GATTGAGGTT	AACCANATTG
ANGTAACAT	CTTCAACACAA	GTGTCCTTAT	GCTGGATGAA
ATTNAGCCCA	CNGGACACCA	NAAAAGAATT	NCCNCTGGTT
CTNNCGGGGG	NCCCCNNNA	CGNNNTNTCC	CCTINTCTCN
NNNGCGGNGA	AGTTNNNNCC	CCCCACTNNAN	NTCTTCCTTC
AANANNTTTC	CNCNNNAGA	GGTTTTCCCN	3'

77. The nucleotide base sequences of ROD PSL SLMB comprises: (748 BP)

5'			
CCTGGTAGGG	TTCCCCGTCA	ACTTCCTCAC	ACTGTACCTC
ACNTTCGAGC	ACAAGAAGCT	ACTAACCCCC	TTAAACTACA
TCCTGCTCAA	CCTGGCGGTG	GGAGACCTCC	TGATGGTGA
AGGAGGGTTC	ACCACCCACA	TCTACACCTC	CATGCACCGC
TAACCTCGTCC	TAGGAAACT	GGGCTGCGCC	ATCGAAGGTT

TCATGGCCAC	CCATGGTGGT	CAGGTCGCC	TITGGTCCT
GGTGTGTTTG	GCCGTGAAA	GGTGGCTGGT	CGTCTGCAAN
CCCATCTCCA	GCTTCGCTT	CCAGGAGTCC	CACTCCCTCA
TGGGCTGGC	CGTGACCTGG	GTGATGGCGA	CGGCTTGTTC
TGTCCCCCCC	CTGGGTGGC	TGGTCTCGCT	ACATCCCAGA
AGGCATGAG	TGCTCATGCG	GAATGGACTA	CTACACTCCC
GCGCCGGCG	TCAACAATGA	ATCCTACGTN	GTGTACATGT
TCNTCANAAA	AANAATNGGA	CCNCNGGGCG	ATCATTTGN
TANGNNAAAGG	CCAGNTGNTG	NGAGCAGTCA	AGGCGGCCG
CGCCGCCAG	CAAGAGTCG	AGACCACCCA	GAGGGCCGAG
AGGAAAGTCA	CCCGNATGGT	NATNANGATG	GTNATNTCNT
TCNTGGTAAG	NAGGGNGCCA	NACGCCAGCG	TGGCCTGGTG
GATCTTNNGN	AACCAGGGNG	CAGAAATTAGG	CCCNGTNTTC
ATGACCCCTGC	CGGCNTTCTT	TGCCAAGA	3'

78. A method as claimed in claim 1 wherein FORWARD (L) primers of CYT b gene region for myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising:

5' CAA CCT CAT CTG TCG TAA AC 3'

and having the following characteristics:

- i. is a 20-mer DNA oligonucleotide (sense),
- ii. has melting temperature of 56.4 degree celius,
- iii. has a molecular weight of 6101.0,
- iv. has no hairpin loops,
- v. has no single dimers,
- vi. has no other dimers,
- vii. has no single bulge loops or internal loops, and
- viii. has no palindromes.

79. A method as claimed in claim 1 wherein BACKWARD (H) primer of CYT b gene region for myctophid fish *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising :

5' GCT CGG GCT GCT GGA ATC TT 3'

and having the following characteristics:

- i. is a 20-mer DNA

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- ii. is an antisense oligonucleotide
 - iii. has a melting point of 70.8 degree celcius.
 - iv. has a molecular weight of 6220.1.
 - v. has no hairpin loops, no single bulge loops, no other internal loops, no single internal loops, no other bulge loops or palindromes.
 - vi. no single dimers or other dimers.
80. A method as claimed in claim 1 wherein forward primer of ITS2 F gene region for myctophid fish *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:
5' ACT TGA CTG ACC TTC TTA CT 3'
and having the following characteristics:
 - i. is a 20-mer sense oligonucleotide,
 - ii. has a melting point of 51.3 degree celcius,
 - iii. has a molecular weight of 6098.0,
 - iv. has no palindromes, loops and dimers,
81. A method as claimed in claim 1 wherein forward primer of ITS2 H gene region for myctophid fish *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising :
5' ATA CTC TGC GGA CAT ACT TGA CTG 3'
and having the following characteristics:
 - i. is a 24-mer antisense oligonucleotide,
 - ii. has a melting point of 65.4 degree celcius.
 - iii. has a molecular weight of 7407.9.
 - iv. has no palindromes, loops and dimers.
82. A method as claimed in claim 1 wherein forward primer of pro-L for myctophid fish *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:
5' CAG TCT CGT CAA ACC AAG TCA AAC 3'
and having the following characteristics:

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- i. is a 24-mer sense oligonucleotide
 - ii. has a melting point of 67.8 degree celcius.
 - iii. has a molecular weight of 7354.9.
 - iv. has no palindromes, loops and dimers.
83. A method as claimed in claim 1 wherein backward primer for Dloop for mitochondrial control region (dloop H) gene region for myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising :
- 5' ATA ATC ATC CAG CAT AAA CAC AC 3'
- and having the following characteristics:
- i. is a 23-mer antisense oligonucleotide,
 - ii. has a melting point of 61.2 degree celcius.
 - iii. has a molecular weight of 7033.7.
 - iv. has no palindromes, loops and dimers.
84. A method as claimed in claim 1 wherein the FORWARD primer (ROD- L) for Rhodopsin gene region of myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising:
- 5' CCT GGT AGA GTT CGC CGT CA 3'
- and having the following characteristics:
- i. is a 20-mer sense oligonucleotide
 - ii. has a melting point of 67.4 degree celcius.
 - iii. has a molecular weight of 6189.0.
 - iv. has no palindromes, loops and dimers.
85. A method as claimed in claim 1 wherein the backward primer (ROD- H) for Rhodopsin gene region of myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising:
- 5' CGT GTT CCT TAT CAT TGT GCC T 3'
- and having the following characteristics:
- i. is a 22-mer antisense oligonucleotide

- ii. has a melting point of 66.4 degree celcius.
iii. has a molecular weight of 6738.4.
iv. has no palindromes, loops and dimers.

86. A method as claimed in claim 1 wherein the forward primer of 16S-L of the myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising:
5' CAC CAG CCA AGT ATG TTT CTC 3'
and having the following characteristics:
i. is a 21-mer sense oligonucleotide
ii. has a melting point of 61.5 degree celcius.
iii. has a molecular weight of 6421.4.
iv. has no palindromes, loops and dimers.

87. A method as claimed in claim 1 wherein the backward primer of 16s rRNA of myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising:
5' TCG TAG TTC AGC AGT CAG 3'
and having the following characteristics:
i. is a 18-mer antisense oligonucleotide
ii. has a melting point of 51.2 degree celcius.
iii. has a molecular weight of 5594.7.
iv. has no palindromes, hairpin loops and dimers.

88. A method as claimed in claim 1 wherein the forward primer 16S-L of myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising:
5' CTA TTC GCC TCG CTC AGA C 3'
and having the following characteristics:
i. is a 19-mer sense oligonucleotide
ii. has a melting point of 62.1 degree celcius.
iii. has a molecular weight of 5779.8.
iv. has no palindromes, hairpin loops and dimers.

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89. A method as claimed in claim 1 wherein a primer 12S-H for *Lampanyctus regalis* (LRMB) is an oligonucleotide comprising:
5' GCC TCC ATC ATC CCT CAC CTT AC 3'
and having the following characteristics:
i. is a 23-mer antisense oligonucleotide
ii. has a melting point of 70.8 degree celcius.
iii. has a molecular weight of 6895.5
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
90. A method as claimed in claim 1 wherein the primer 12S-L for *Lampanyctus regalis* (LRMB) is an oligonucleotide comprising:
5' CTA TTC GCC TCG CTC AGA C 3'
and having the following characteristics:
i. is a 19-mer sense oligonucleotide
ii. has a melting point of 62.1 degree celcius.
iii. has a molecular weight of 5779.8
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
91. A method as claimed in claim 1 wherein 16S-L forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' AAA TCC GCC CTT ATG TGT GTT C 3'
and having the following characteristics:
i. is a 22-mer sense oligonucleotide
ii. has a melting point of 67.9 degree celcius.
iii. has a molecular weight of 6756.4
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

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92. A method as claimed in claim 1 wherein 16S-H backward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' CTC CGT CCG TCT CGC CTC TG 3'
and having the following characteristics:
i. is a 20-mer antisense oligonucleotide
ii. has a melting point of 71.7 degree celcius.
iii. has a molecular weight of 6052.0
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
93. A method as claimed in claim 1 wherein 12S-H forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' CAT CGG CTT GCT CTA TTC CTT G 3'
and having the following characteristics:
i. is a 22-mer antisense oligonucleotide
ii. has a melting point of 68.8 degree celcius.
iii. has a molecular weight of 6723.4
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
94. A method as claimed in claim 1 wherein 12S-L forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising:
5' TCT ATC GGC GGC GTA TCA C 3'
and having the following characteristics:
i. is a 19-mer sense oligonucleotide
ii. has a melting point of 65.8 degree celcius.
iii. has a molecular weight of 5859.8
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

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95. A method as claimed in claim 1 wherein 16S-H primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' GGC GAT TCT ACG GCA CGG GCG 3'
and having the following characteristics:
i. is a 21-mer antisense oligonucleotide
ii. has a melting point of 80.4 degree celcius.
iii. has a molecular weight of 6568.3
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
96. A method as claimed in claim 1 wherein 16S-L forward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' AAA CTG GTC CTC AAC TAT GTC A 3'
and having the following characteristics:
i. is a 22-mer sense oligonucleotide
ii. has a melting point of 60.7 degree celcius.
iii. has a molecular weight of 6758.5
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
97. A method as claimed in claim 1 wherein 16S-H backward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' GGC GAT TCT ACG GCA CGG GCG 3'
and having the following characteristics:
i. is a 21-mer antisense oligonucleotide
ii. has a melting point of 80.4 degree celcius.
iii. has a molecular weight of 6568.3
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

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98. A method as claimed in claim 1 wherein 12S-H backward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' CCG ATT CAG CCA CGA TTC CCT C 3'
and having the following characteristics:
i. is a 22-mer antisense oligonucleotide
ii. has a melting point of 74.6 degree celcius.
iii. has a molecular weight of 6671.4
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
99. A method as claimed in claim 1 wherein 12S-L forward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising:
5' CCT AAA GCC CAG ATA ACT ACA 3'
i. is a 21-mer sense oligonucleotide
ii. has a melting point of 59.2 degree celcius.
iii. has a molecular weight of 6432.3
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
100. A method as claimed in claim 1 wherein 16S-H backward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:
5' CGT GTT CTG ATG ATG ATG TGC T 3'
i. is a 22-mer antisense oligonucleotide
ii. has a melting point of 64.7 degree celcius.
iii. has a molecular weight of 6867.5
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
101. A method as claimed in claim 1 wherein 16S-L forward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:
5' ATT CCT TCC TCT TAG TAT G 3'

- i. is a 19-mer sense oligonucleotide
ii. has a melting point of 49.5 degree celcius.
iii. has a molecular weight of 5799.8
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
102. A method as claimed in claim 1 wherein 12S-H backward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:
5' GCT GAA CTT ACT ATG CCC TAC T 3'
i. is a 22-mer antisense oligonucleotide
ii. has a melting point of 60.3 degree celcius.
iii. has a molecular weight of 6725.4
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
103. A method as claimed in claim 1 wherein 12S-L forward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising:
5' CCG ATT GAC GCC GAA CTA TG 3'
i. is a 20-mer sense oligonucleotide
ii. has a melting point of 68.1 degree celcius.
iii. has a molecular weight of 6182.1
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
104. A method as claimed in claim 1 wherein 16S-H backward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:
5' TAC GCA TAA CGG CTC TGG 3'
i. is a 18-mer DNA oligonucleotide (Antisense)
ii. has a melting point of 61.4 degree celcius.
iii. has a molecular weight of 5579.7

- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
105. A method as claimed in claim 1 wherein 16S-L forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:
5' CTA CTA CAC CTC AAC TAC ATC T 3'
i. is a 22-mer sense oligonucleotide
ii. has a melting point of 52.4 degree celcius.
iii. has a molecular weight of 6638.4
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
106. A method as claimed in claim 1 wherein 12S-H forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:
5' CCC ACT CAC TGC TAA CTC C 3'
i. is a 19-mer sense oligonucleotide
ii. has a melting point of 58.4 degree celcius.
iii. has a molecular weight of 5708.8
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.
107. A method as claimed in claim 1 wherein 12S-L forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising:
5' GGC TAA CTA CAA TCA TCT GCT 3'
i. is a 21-mer sense oligonucleotide
ii. has a melting point of 58.5 degree celcius.
iii. has a molecular weight of 6445.2
iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Analysis of "table1 (slmb primer cyt L)" a 20-mer DNA Oligonucleotide (Sense)

5 . CAA CCT CAT CTG TCG TAA AC 3 .

Oligonucleotide Analysis

	6101.0	25.0 degrees C
Molecular weight	56.4	Delta G Temperature
Tm thermodynamic	48.8	C Probe concentration
Filter Tm	66.2	C Salt concentration
% GC Tm	58.0	C Formamide concentration
AT+GC Tm	5.3	3' End length
Absorbance	32.5	Run length
Absorbance	45.0	Palindrome length
Percent GC	-28.7	Hairpin loop stem length
Delta G	-140.6	
Delta H	-368.0	
Delta S	-5.9	
3' End Delta G		

Structural Analysis Summary

	/	Number of base runs / Palindromes
Number of hairpin loops	/	0 / 0
Number of dimers	/	0 / 0
Number of bulge loops	/	0 / 0
Number of internal loops	/	0 / 0
Number of 2-oligo internals	/	0 / 0

Analysis of "table 2 (slimb primer cyt H)" a 20-mer DNA Oligonucleotide (Antisense)

5' GCT CGG GCT GCT GGA ATC TT 3'

Oligonucleotide Analysis

	Analysis Parameters	
Molecular weight	6220.1	25.0 degrees C
Tm thermodynamic	70.8 degrees	c Probe concentration
Filter Tm	63.2 degrees	c Salt concentration
% GC Tm	72.3 degrees	c Formamide concentration
A+GC Tm	64.0 degrees	c 3' End length
Absorbance	5.6 nmol/A260	Run length
Absorbance	34.8 ug/A260	Palindrome length
Percent GC	60.0 %	Hairpin loop stem length
Delta G	-37.5 kcal/Mol	
Delta H	-164.6 kcal/Mol	
Delta S	-419.9 eu	
3' End Delta G	-5.1 kcal/Mol	

Structural Analysis Summary

Number of base runs	/	palindromes	0 / 0
Number of hairpin loops	/		0 / 0
Number of dimers	/	2-oligo dimers	0 / 0
Number of bulge loops	/	2-oligo bulges	0 / 0
Number of internal loops	/	2-oligo internals	0 / 0

Analysis of "table 3 (slmb primer ITS2 F)" a 20-mer DNA Oligonucleotide (Sense)

5' ACT TGA CTG ACC TTC TTA CT 3'

Oligonucleotide Analysis

	<u>Analysis Parameters</u>	
Molecular weight	6098.0	Delta G Temperature 25.0 degrees C
Tm thermodynamic	51.3 degrees C	Probe concentration 0.6 PMol
Filter Tm	43.7 degrees C	Salt concentration 1000.0 mMol
% GC Tm	64.2 degrees C	Formamide concentration 0.0 %
AT+GC Tm	56.0 degrees C	7 bases
Absorbance	5.6 nmol/A260	3. End length
Absorbance	34.0 ug/A260	Run length
Percent GC	40.0 %	4 bases
Delta G	-26.5 kcal/Mol	Palindrome length
Delta H	-137.7 kcal/Mol	Hairpin loop stem length
Delta S	-365.8 eu	3 bases
[3', End Delta G	-3.9 kcal/Mol	

Structural Analysis Summary

Number of base runs	/	Number of palindromes	0 / 0
Number of hairpin loops	/	Number of 2-oligo dimers	0 / 0
Number of dimers	/	Number of 2-oligo bulges	0 / 0
Number of bulge loops	/	Number of 2-oligo internals	0 / 0
Number of internal loops	/		
Number of 2-oligo			

Analysis of "table 4 (simb primer ITS2-H)" a 24-mer DNA Oligonucleotide (Antisense)

ATA CTC TGC GGA CAT ACT TGA CTC

ATA CTC TGC GGA CAT ACT TGA CTC

Oligonucleotide Analysis		Analysis Parameters		
Molecular weight	7407.9	Delta G Temperature	25.0 degrees C	
Tm thermodynamic	65.4 degrees C	Probe concentration	0.6 pMol	
Filter Tm	57.8 degrees C	Salt concentration	1000.0 uMol	
% GC Tm	72.2 degrees C	Formamide concentration	0.0 %	
A+G+C Tm	70.0 degrees C	3' End length	7 bases	
Absorbance	4.4 nmol/A260	Run length	4 bases	
Absorbance	32.4 ug/A60	Palindrome length	8 bases	
Percent GC	45.8 %	Hairpin loop stem length	3 bases	
Delta G	-35.5 kcal/mol			
Delta H	-169.5 kcal/mol			
Delta S	-442.0 eu			
3' End Delta G	-5.2 kcal/mol			

Structural Analysis Summary		
Number of base runs	/	Number of palindromes
Number of hairpin loops		0 / 0
Number of dimers		0 / 0
Number of bulge loops		2-oligo bulges
Number of internal loops		2-oligo internals

3'

Analysis of "table 5 (slmb primer pro-L) " a 24-mer DNA Oligonucleotide(Sense)

CAG TCT CGT CAA ACC ACG TCA AAC

Oligonucleotide Analysis		Analysis Parameters	
Molecular weight	7354.9	Delta G Temperature	25.0 degrees C
Tm thermodynamics	67.8 degrees	C Probe concentration	0.6 pmol
Filter Tm	60.2 degrees	C Salt concentration	1000.0 mMol
% GC Tm	72.2 degrees	C Formamide concentration	0.0 %
A-T/G-C Tm	70.0 degrees	C 3' End length	7 bases
Absorbances	4.3 nmol/A260	Run length	
Absorbance	31.4 ug/A260	Palindrome length	4 bases
Percent GC	45.8 %	Hairpin length	8 bases
Delta G	-36.5 kcal/Mol	Hairpin loop stem length	3 bases
Delta H	-169.9 kcal/Mol		
Delta S	-439.7 eu		
3' End Delta G	-4.9 kcal/Mol		

Structural Analysis Summary

Number of base runs	/	palindromes	0 / 0
Number of hairpin loops			
Number of dimers	/	2-oligo dimers	0 / 0
Number of bulge loops	/	2-oligo bulges	0 / 0
Number of internal loops	/	2-oligo internals	0 / 0

Analysis of "table 6 (slmb primer Dloop-H)" a 23-mer DNA Oligonucleotides (Antisense)

Oligonucleotide Analysis		Analysis Parameters	
Oligonucleotide	Sequence	Parameter	Value
Molecular weight	7033.7	Delta G Temperature	25.0 degrees C
Tm thermodynamic	61.2 degrees C	Probe concentration	0.6 pmol
Filter Tm	53.6 degrees C	Salt concentration	1000.0 mMol
% GC Tm	66.4 degrees C	Formamide concentration	0.0 %
AT+GC Tm	62.0 degrees C	3' End Length	7 bases
Absorbance	4.3 nmol/A260	Run length	4 bases
Absorbance	30.0 ug/A260	Palindrome length	8 bases
Percent GC	34.8 %	Hairpin loop	3 bases
Delta G	-32.9 kcal/Mol	stem length	3 bases
Delta H	-163.3 kcal/Mol		
Delta S	-429.7 au		
3' End Delta G	-4.6 kcal/Mol		

Structural Analysis Summary

Number of base runs	/	palindromes	0 / 0
Number of hairpin loops	/		
Number of dimers	/	2-oligo dimers	0 / 0
Number of bulge loops	/	2-oligo bulges	0 / 0
Number of internal loops	/	2-oligo internals	0 / 0

Analysis of "table 7 (slmb primer R0D-L)" a 20-mer DNA Oligonucleotide (Sense)

Oligonucleotide Analysis	
Molecular weight	6189.0
Tm thermodynamic	67.4 degrees C
Filter Tm	59.8 degrees C
% GC Tm	72.3 degrees C
AT+GC Tm	64.0 degrees C
Absorbance	5.3 nmol/A260
Absorbance	33.0 ug/A260
Percent GC	60.0 %
Delta G	-34.7 kcal/Mol
Delta H	-154.3 kcal/Mol
Delta S	-394.4 eu
3' End Delta G	-9.6 kcal/Mol

Analysis Parameters	
Delta G	25.0 degrees C
Temperature	0.6 PMol
Probe concentration	1000.0 mMol
Salt concentration	0.0 %
Formamide concentration	7 bases
3' End length	7 bases
Run length	4 bases
Palindrome length	8 bases
Hairpin loop stem length	3 bases

Structural Analysis Summary	
Number of base runs	/ palindromes
Number of hairpin loops	0 / 0
Number of dimers	0 / 0
Number of bulge loops	0 / 0
Number of internal loops	0 / 0
Number of 2-oligo internals	0 / 0

Analysis of "table 8 (simb primer ROD-H)" a 22-mer DNA Oligonucleotide (Antisense)

	CGT	GTT	CCT	TAT	CAT	TGT	GCC	T	3'
Oligonucleotide Analysis									
Analysis Parameters									
Molecular weight	6738.4								
Delta G thermodynamic	66.4 degrees	C	Delta G Temperature	25.0 degrees C					
Filter Tm	58.8 degrees	C	Probe concentration	0.6 pmol					
% GC Tm	69.5 degrees	C	Salt concentration	1000.0 mMol					
AT+GC Tm	64.0 degrees	C	Formamide concentration	0.0 %					
Absorbance	5.2 nmol/A260	C	3' End length	7 bases					
Absorbance	34.9 ug/A260	C	Run length	4 bases					
Percent GC	45.5 %	kcal/Mol	Palindrome length	8 bases					
Delta G	-35.4 kcal/Mol		Hairpin loop stem length	3 bases					
Delta H	-16.0 kcal/Mol								
Delta S	-427.3 eu								
3' End Delta G	-7.9 kcal/Mol								

	Structural Analysis Summary	
Number of base runs	/	palindromes
Number of hairpin loops	0	/ 0
Number of dimers	0	/ 0
Number of bulge loops	0	/ 0
Number of internal loops	0	/ 0
Number of 2-oligo internals	0	/ 0

Analysis of "table 9 (LRMB primer 16S-L)" a 21-mer DNA Oligonucleotide(Sense)

5' CAC CAG CCA AGT ATG TTT CTC 3'

Oligonucleotide Analysis

Molecular weight		6421.2	Analysis Parameters	
Tm thermodynamic	61.5 degrees C	Delta G Temperature	25.0 degrees C	
Filter Tm	53.9 degrees C	Probe concentration	0.6 pMol	
% GC Tm	68.9 degrees C	Salt concentration	1000.0 mMol	
AT+GC Tm	62.0 degrees C	Formamide concentration	0.0 %	
Absorbance	62.0 nmol/A260	Run Length	7 bases	
Absorbance	5.1 nmol/A260	Palindrome length	4 bases	
Percent GC	33.0 %	Hairpin loop length	8 bases	
Delta G	47.6 %	Hairpin loop stem length	3 bases	
Delta H	-31.9 kcal/Mol			
Delta S	-152.3 kcal/Mol			
3. End Delta G	-396.4 eu			
	-4.9 kcal/Mol			

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		
Number of dimers	/ 2-oligo dimers	0 / 0
Number of bulge loops	/ 2-oligo bulges	0 / 0
Number of internal loops	/ 2-oligo internals	0 / 0

Analysis of "table 10 (LRMB primer 16S-H)" a 18-mer DNA Oligonucleotide(Antisense)

5' TCG TAG TTC AGC AGT CAG 3'

Oligonucleotide Analysis

	Analysis Parameters	
Molecular weight	5594.7	Delta G Temperature 25.0 degrees C
Tm thermodynamic	51.2 degrees	Probe concentration 0. pMol
Filter Tm	43.6 degrees	Salt concentration 1000.0 mMol
% GC Tm	64.5 degrees	Formamide concentration 0.0 %
AT+GC Tm	54.0 degrees	C' End length 7 bases
Absorbance	5.7 mMol/A260	Run length 4 bases
Absorbance	31.8 ug/R260	Palindrome length 8 bases
Percent GC	50.0 %	Hairpin loop stem length 3 bases
Delta G	-25.3 kCal/Mol	
Delta H	-123.0 kCal/Mol	
Delta S	-320.5 eu	
3. End Delta G	-4.9 kCal/Mol	

Structural Analysis Summary

Number of base runs	/	palindromes	0 / 0
Number of hairpin loops	/	2-oligo dimers	0 / 0
Number of dimers	/	2-oligo bulges	0 / 0
Number of bulge loops	/	2-oligo internals	0 / 0
Number of internal loops	/		

Analysis of "table 11 (LRN8 primer 12S-L)" a 19-mer DNA Oligonucleotide (Sense)

5' C'TA TTC GCC TCG CTC AGA C 3'

Oligonucleotide Analysis

	5779.8	5779.8	Delta G Temperature 25.0 degrees C	Analysis Parameters
Molecular weight	5779.8	62.1 degrees C	Delta G Temperature	25.0 degrees C
Tm thermodynamic	54.5 degrees C	Probe concentration	0.6 pMol	
Filter Tm	69.7 degrees C	Salt concentration	1000.0 mMol	
% GC Tm	60.0 degrees C	Formamide concentration	0.0 %	
AT+GC Tm	66.0 nmol/A260	3' End length	7 bases	
Absorbance	34.6 ug/A260	Run length	4 bases	
Absorbance	57.9 %	Palindrome length	8 bases	
Percent GC	-31.8 kcal/Mol	Hairpin loop stem length	3 bases	
Delta G	-146.6 kcal/Mol			
Delta H	-378.6 eu			
Delta S	-4.6 kcal/Mol.			
3' End Delta G				

Structural Analysis Summary

Number of base runs / Palindromes	0 / 0
Number of hairpin loops	0
Number of dimers / 2-oligo dimers	0 / 0
Number of bulge loops / 2-oligo bulges	0 / 0
Number of internal loops / 2-oligo internals	0 / 0

Analysis of "table 12 (LRMB primer 12S-H)" a 23-mer DNA Oligonucleotide (Antisense)

	GCC	TCC	ATC	ATC	CCT	CAC	CTT	AC	3'
--	-----	-----	-----	-----	-----	-----	-----	----	----

Oligonucleotide Analysis		Analysis Parameters							
Molecular weight	6895.5	Delta G	Temperature	25.0	degrees C				
Tm thermodynamic	70.8	Concentration	0.6	pMol					
Filter Tm	63.2	Probe concentration	1000.0	nMol					
% GC Tm	75.3	Salt concentration	0.0	%					
AT+GC Tm	72.0	Formamide concentration	7	bases					
Absorbance	5.1	End length	4	bases					
Absorbance	34.9	Run length	8	bases					
Percent GC	56.5	Palindrome length	3	bases					
Delta G	-38.9	Hairpin loop stem length	3	bases					
Delta H	-174.6								
Delta S	-448.9								
3' End Delta G	-5.1	kCal/Mol							

Structural Analysis Summary

	/	
Number of base runs	/	palindromes
Number of hairpin loops	/	
Number of dimers	/	2-oligo dimers
Number of bulge loops	/	2-oligo bulges
Number of internal loops	/	2-oligo internals

Analysis of "table 13 (DTMB primer 16S-H)" a 20-mer DNA Oligonucleotide (Antisense)

5' CTC CGT CCG TCT CGC CTC TG 3'

Oligonucleotide Analysis		Analysis Parameters	
Molecular weight	6052.0	Delta G Temperature	25.0 degrees C
Tm thermodynamic	71.7 degrees C	Probe concentration	0.6 pMol
Filter Tm	64.1 degrees C	Salt concentration	1000.0 mMol
% GC Tm	76.4 degrees C	Formamide concentration	0.0 %
A+GC Tm	68.0 degrees C	3' End length	7 bases
Absorbance	6.1 nmol/A260	Run length	4 bases
Absorbance	37.2 ug/A260	Palindrome length	8 bases
Percent GC	70.0 %	Hairpin loop stem length	3 bases
Delta G	-37.1 kCal/Mol		
Delta H	-157.8 kCal/Mol		
Delta S	-398.9 eu		
3' End Delta G	-7.9 kcal/Mol		

Structural Analysis Summary

Structural Analysis Summary	
Number of base runs	/ palindromes
Number of hairpin loops	0 / 0
Number of dimers	/ 2-oligo dimers
Number of bulge loops	/ 2-oligo bulges
Number of internal loops	/ 2-oligo internals
Number of	0 / 0

Analysis of "table 14 (DTMB primer 16S-L)" a 22-mer DNA Oligonucleotide(Sense)

5' AAA TCC GCC CTT ATG TGT GTC 3'

Oligonucleotide Analysis

	Analysis Parameters	
	25.0 degrees C	0.6 pMol
Molecular Weight	6756.4	C
Tm thermodynamic	67.9 degrees C	Probe concentration
Filter Tm	60.3 degrees C	Salt concentration
% GC Tm	69.5 degrees C	Formamide concentration
AT+GC Tm	64.0 degrees C	0.0 %
Absorbance	4.9 nmol/A260	3' End length
Absorbance	33.3 ug/A260	Run Length
Percent GC	45.5 %	Palindrome length
Delta G	-36.9 kcal/Mol	Hairpin loop stem length
Delta H	-171.5 kcal/Mol	
Delta S	-444.2 eu	
3' End Delta G	-4.9 kcal/Mol	

Structural Analysis Summary

Number of base runs / Palindromes	0 / 0
Number of hairpin loops	0
Number of dimers	/ 0
Number of bulge loops	/ 0
Number of internal loops	/ 0
Number of 2-oligo internals	0 / 0

Analysis of "table 15 (DTM primer 12S-H)" a 22-mer DNA Oligonucleotide(Antisense)

	CAT	CGG	CTT	GCT	CTA	TTC	CTT	G	3'
Oligonucleotide Analysis									
Molecular weight									
Molar weight	6723.4								
Tm thermodynamic	68.4 degrees	c							
Filter Tm	61.2 degrees	c							
% GC Tm	71.3 degrees	c							
AT+GC Tm	66.0 degrees	c							
Absorbance	5.0 nmol/A260	c							
Absorbance	35.5 ug/A260	c							
Percent GC	50.0 %								
Delta G	-37.5 kcal/Mol								
Delta H	-172.0 kcal/Mol								
Delta S	-444.3 eu								
3' End Delta G	-7.0 kcal/Mol								

	Analysis Parameters			25.0 degrees C
Delta G Temperature	68.4 degrees	c		0.6 pmol
Probe concentration	61.2 degrees	c		1000.0 nMol
Salt concentration	71.3 degrees	c		0.0 %
Formamide concentration	66.0 degrees	c		7 bases
3' End length	5.0 nmol/A260	c		4 bases
Run length	35.5 ug/A260	c		8 bases
Palindrome length				Hairpin loop stem length
				3 bases

Structural Analysis Summary

	Number of base runs / palindromes	0 / 0
Number of hairpin loops	0	0
Number of dimers	0	0
Number of bulge loops / 2-oligo bulges	0 / 0	0 / 0
Number of internal loops / 2-oligo internals	0 / 0	0 / 0

Analysis of "table 16 (DTMB primer 12S-L)" a 19-mer DNA Oligonucleotide (Sense)

5' TCT ATC GGC GGC GTA C 3'

Oligonucleotide Analysis

Molecular weight	5859.8	Analysis Parameters	
Tm thermodynamic	65.8 degrees	Delta G Temperature	25.0 degrees C
Filter Tm	58.8 degrees	c Probe concentration	0.16 pMol
% GC Tm	58.2 degrees	c Salt concentration	1.000.0 mMol
AT+GC Tm.	69.7 degrees	c Formamide concentration	0.0 %
Absorbance	60.0 degrees	c 3' End length	7 bases
Absorbance	5.7 nmol/A260	c Run length	4 bases
Percent GC	33.4 ug/A260	c Palindrome length	8 bases
Delta G	57.9 %	c Hairpin loop stem length	3 bases
Delta H	-33.9 kCal/mol		
Delta S	-152.5 kCal/mol		
Delta G	-391.2 eu		
	-3.5 kCal/mol		

3' End Delta G

Structural Analysis Summary

Number of base runs	/	Number of palindromes
Number of hairpin loops	/	0 / 0
Number of dimers	/	0 / 0
Number of bulge loops	/	0 / 0
Number of internal loops	/	0 / 0
Number of 2-oligo bulges	/	0 / 0
Number of 2-oligo internals	/	0 / 0

Analysis of "table 17 (TCM primer 16S-H)" a 21-mer DNA Oligonucleotide (Antisense)

5' GGC GAT TCT ACG GCA CGG GCG 3'

Oligonucleotide Analysis

	Analysis Parameters		
Molecular weight	6568.3	Delta G	25.0 degrees C
Tm thermodynamic	80.4 degrees C	Temperature	0.6 pmol
Filter Tm	72.8 degrees C	Probe concentration	1000.0 mMol
% GC Tm	78.6 degrees C	Salt concentration	0.0 %
AT+GC Tm	72.0 degrees C	Formamide concentration	0.0
Absorbance	3', End length	Run length	7 bases
Absorbance	5.1 nMol/A260	Palindrome length	4 bases
Percent GC	33.3 ug/A260	Hairpin loop stem length	8 bases
Delta G	71.4 %		3 bases
Delta H	-44.7 kcal/Mol		
Delta S	-186.4 kcal/Mol		
3' End Delta G	-468.6 eu		
	-12.8 kcal/Mol		

Structural Analysis Summary

Number of base runs	/ palindromes	0 / 0
Number of hairpin loops		
Number of dimers	/ 2-oligo	0 / 0
Number of bulge loops	/ 2-oligo	0 / 0
Number of internal loops	/ 2-oligo	0 / 0
Number of internals		

5 · AAA CTC GTC AAC TAT GTC A 3 ·

Analysis of "table 18 (TCMB primer 16S-L)" a 22-mer DNA Oligonucleotide(Sense)

Oligonucleotide Analysis		Analysis Parameters	
Molecular weight	6758.5	Delta G Temperature	25.0 degrees C
Tm thermodynamic	60.7 degrees C	Probe concentration	0.6 pMol
Filter Tm	53.1 degrees C	Salt concentration	1000.0 mMol
% GC Tm	67.6 degrees C	Formamide concentration	0.0 %
AT+GC Tm	62.0 degrees C	3' End length	7 bases
Absorbance	4.7 nmol/A260	Run length	4 bases
Absorbance	31.7 ug/A260	Palindrome length	8 bases
Percent GC	40.9 %	Hairpin loop stem length	3 bases
Delta G	-31.7 kcal/Mol		
Delta H	-153.3 kcal/Mol		
Delta S	-400.5 au		
	-4.1 kcal/Mol		
3' End Delta G			

Structural Analysis Summary

Number of base runs	/	palindromes	0 / 0
Number of hairpin loops	/	dimers	0 / 0
Number of dimers	/	2-oligo dimers	0 / 0
Number of bulge loops	/	2-oligo buters	0 / 0
Number of internal loops	/	2-oligo internals	0 / 0

Analysis of "table 19 (TCGA primer 12S-H)" a 22-mer DNA Oligonucleotide (Antisense)

Oligonucleotide Analysis		Analysis Parameters	
Molecular weight	6671.4	Delta G Temperature	25.0 degrees C
Tm thermodynamic	74.6 degrees	c probe concentration	0.6 pmol
Filter Tm	67.0 degrees	c salt concentration	1000.0 mMol
% GC Tm	75.0 degrees	c formamide concentration	0.0 %
AT+GC Tm	70.0 degrees	c End length	7 bases
Absorbance	5.1 nmol/A260	Run length	4 bases
Absorbance	34.2 ug/A260	Palindrome length	8 bases
Percent GC	59.1 %	Hairpin loop stem length	3 bases
Delta G	-40.8 kCal/Mol		
Delta H	-176.0 kCal/Mol		
Delta S	-447.5 nJ		
3' End Delta G	-7.9 kcal/mol		

Structural Analysis Summary

Number of base runs	/	Palindromes	0 / 0
Number of hairpin loops	/	2-oligo dimers	0 / 0
Number of dimers	/	2-oligo bulges	0 / 0
Number of bulge loops	/	2-oligo internals	0 / 0
Number of internal loops	/		
Number of 3' overhangs	/		

Analysis of "table 20 (TMB primer 12S-L)" a 21-mer DNA Oligonucleotide (Sense)

5' CCT AAA GCC CAG ATA ACT ACA 3'

Oligonucleotide Analysis		Analysis Parameters	
Molecular weight	6432.3	Delta G	25.0 degrees C
Tm thermodynamic	59.2 degrees	Temperature	0.6 pmol
Filter Tm	51.6 degrees	probe concentration	1000.0 mMol
% GC Tm	66.9 degrees	salt concentration	0.0 %
AT+GC Tm	60.0 degrees	formamide concentration	7 bases
Absorbance	4.8 nmol/A260	3' End length	4 bases
Absorbance	30.6 ug/A260	Run length	8 bases
Absorbance	42.9 %	Palindrome length	3 bases
Absorbance	-31.7 kcal/Mol	Hairpin loop stem length	
Percent GC	-159.4 kcal/Mol		
Delta G	-421.0 eu		
Delta H			
Delta S	-3.9 kcal/Mol		
3' End Delta G			

Structural Analysis Summary

Number of base runs	/	palindromes	0 / 0
Number of hairpin loops	/	2-oligo dimers	0 / 0
Number of dimers	/	bulges	0 / 0
Number of bulge loops	/	2-oligo internals	0 / 0
Number of internal loops	/	2-oligo	

Analysis of "table 21 (PCMB primer 16S-H)" a 22-mer DNA Oligonucleotide (Antisense)

5' CGT GTT CTC ATG ATG ATG TGC T 3'

Oligonucleotide Analysis		Analysis Parameters	
Molecular weight	6867.5	Delta G Temperature	25.0 degrees C
Tm thermodynamic	64.7 degrees	Probe concentration	0.6 pMol
Filter Tm	57.1 degrees	C Salt concentration	1000.0 mMol
% GC Tm	69.5 degrees	C Formamide concentration	0.7 bases
AT+GC Tm	64.0	C 3' End length	4 bases
Absorbance	4.9 nmol/A260	Run length	8 bases
Absorbance	33.4 ug/A260	Palindrome length	3 bases
Percent GC	45.5 %	Hairpin loop stem length	
Delta G	-33.0 kcal/Mol		
Delta H	-150.2 kcal/Mol		
Delta S	-385.9 eu		
3' End Delta G	-6.3 kcal/Mol		

Structural Analysis Summary

Number of base runs	/	Number of palindromes	0 / 0
Number of hairpin loops	/	Number of dimers	0 / 0
Number of dimers	/	Number of bulges	0 / 0
Number of bulge loops	/	Number of 2-oligo internals	0 / 0
Number of internal loops	/	Number of 2-oligo internals	0 / 0

Analysis of "table 22 (PCMB primer 16S-L)" a 19-mer DNA Oligonucleotide (Sense)

5' ATT CCT TCC TCT TAG TAT G 3'

Oligonucleotide Analysis

Molecular weight	5799.8	Analysis Parameters	
Tm thermodynamic	49.5 degrees	Delta G Temperature	25.0 degrees C
Filter Tm	41.9 degrees	C Probe concentration	0.6 pmol
% GC Tm	61.1 degrees	C Salt concentration	100.0 mMol
AT+GC Tm	52.0 degrees	C Formamide concentration	0.0 %
Absorbance	5.8 nMol/A260	C End length	7 bases
Absorbance	33.6 ug/A260	Run Length	4 bases
Percent GC	36.8 %	Palindrome length	8 bases
Delta G	-26.1 kcal/mol	Hairpin loop stem length	3 bases
Delta H	-138.8 kcal/mol		
Delta S	-371.5 eu		
3. End Delta G	-3.1 kcal/mol		

Structural Analysis Summary

Number of base runs / palindromes	0 / 0
Number of hairpin loops	0 / 0
Number of dimers / 2-oligo dimers	0 / 0
Number of bulge loops / 2-oligo bulges	0 / 0
Number of internal loops / 2-oligo internals	0 / 0

Analysis of 'table 23 (PCMB primer 12S-H)" a 22-mer DNA Oligonucleotide (Antisense)

5' GCT GAA CTT ACT ATG CCC TAC T 3'

Oligonucleotide Analysis		Analysis Parameters	
Molecular weight	6725.4	Delta G Temperature	25.0 degrees C
Tm thermodynamic	60.3 degrees C	C Probe concentration	0.6 pmol
Filter Tm	52.7 degrees C	C Salt concentration	1000.0 nmol
% GC Tm	69.5 degrees C	C Formamide concentration	0.0 %
AT+GC Tm	64.0 degrees C	C End length	7 bases
Absorbance	5.0 nmol/A260	Run length	4 bases
Absorbance	33.6 ug/A260	Palindrome length	8 bases
Percent GC	45.5 %	Hairpin loop stem length	3 bases
Delta G	-32.7 kcal/Mol		
Delta H	-164.7 kcal/Mol		
Delta S	-435.2 eu		
Delta G	-6.6 kcal/Mol		

(3. End Delta G

Structural Analysis Summary

Number of base runs	/	Number of palindromes	/
Number of hairpin loops		0	/ 0
Number of dimers		0	/ 0
Number of bulge loops		0	/ 0
Number of internal loops	/	0	/ 0
Number of 2-oligo internals		0	/ 0

Analysis of "table 24 (PCMB primer 12S-L)" a 20-mer DNA Oligonucleotide (Sense)

5' CCG ATT GAC GCC GAA CTA TG 3'

Oligonucleotide Analysis		Analysis Parameters	
Molecular weight	6182.1	Delta G Temperature	25.0 degrees C
Tm thermodynamic	68.1 degrees C	Probe concentration	0.6 pMol
Filter Tm	60.5 degrees C	Salt concentration	1000.0 mMol
% GC Tm	70.3 degrees C	Formamide concentration	0.0 %
AT+GC Tm	62.0 degrees C	End length	7 bases
Absorbance	5.3 nmol/A260	Run length	4 bases
Absorbance	32.5 ug/A260	Palindrome length	8 bases
Percent GC	55.0 %	Hairpin loop stem length	3 bases
Delta G	-35.6 kcal/Mol		
Delta H	-159.4 kcal/Mol		
Delta S	-408.5 eu		
3' End Delta G	-4.1 kcal/Mol		

Structural Analysis Summary

Number of base runs / palindromes	0 / 0
Number of hairpin loops	0
Number of dimers	/ 2-oligo dimers
Number of bulge loops	/ 2-oligo bulges
Number of internal loops	/ 2-oligo internals
	0 / 0

Analysis of "table 25 (S1MB primer 16S-H)" a 18-mer DNA Oligonucleotide (Antisense)

5' TAC GCA TAA CGG CTC TCG 3'

Oligonucleotide Analysis		Analysis Parameters	
Molecular weight	5579.7	Delta G Temperature	25.0 degrees C
Tm thermodynamic	61.4 degrees	c Probe concentration	0.0 nMol
Filter Tm	53.8 degrees	c Salt concentration	1000.0 nMol
% GC Tm	66.8 degrees	c Formamide concentration	0.0 %
AT+GC Tm	56.0 degrees	c 3' End length	7 bases
Absorbance	5.9 nmol/A260	c Run length	4 bases
Absorbance	32.8 ug/A260	c Palindrome length	8 bases
Percent GC	55.6 %	c Hairpin loop stem length	3 bases
Delta G	-31.0 kcal/Mol		
Delta H	-143.5 kcal/Mol		
Delta S	-370.2 eu		
3' End Delta G	-7.9 kcal/Mol		

Structural Analysis Summary

Structural Analysis Summary	
Number of base runs	/ Palindromes
Number of hairpin loops	0 / 0
Number of dimers	/ 2-oligo dimers
Number of bulge loops	/ 2-oligo bulges
Number of internal loops	/ 2-oligo internals

Analysis of "table 26 (SLM primer 16S-L)" a 22-mer DNA Oligonucleotide (Sense)

	CTA	CTA	CAC	CTC	AAC	TAC	ATC	T	3'
Oligonucleotide Analysis									
Analysis Parameters									
Molecular weight	6638.4	degrees C	Delta G Temperature	25.0 degrees C					
Tm thermodynamics	52.4	degrees C	Probe concentration	0.6 pMol					
Filter Tm	44.8	degrees C	Salt concentration	1000.0 mMol					
% GC Tm	67.6	degrees C	Formamide concentration	0.0 %					
AT+GC Tm	62.0	degrees C	3' End length	7 bases					
Absorbance	4.9 nMol/A260		4' End length	4 bases					
Absorbance	32.8 ug/A260		Run length	8 bases					
Percent GC	40.9 %		Palindrome length						
Delta G	-27.6 kcal/Mol		Hairpin loop stem length	3 bases					
Delta H	-146.8 kcal/Mol								
Delta S	-392.2 eu								
3' End Delta G	-3.8 kcal/Mol								

	Structural Analysis Summary
Number of base runs / palindromes	0 / 0
Number of hairpin loops	
Number of dimers	0 / 0
Number of bulge loops	0 / 0
Number of internal loops / 2-oligo internals	0 / 0

Analysis of "table 27 (SLM primer 12S-H)" a 19-mer DNA Oligonucleotide (Antisense)

5' CCC ACT CAC TGC TAA CTC C 3'

Oligonucleotide Analysis

	Analysis Parameters
Molecular weight	5708.8
Tm thermodynamic	58.4 degrees
Filter Tm	50.8 degrees
% GC Tm	69.7 degrees
AT+GC Tm	60.0 degrees
Absorbance	6.1 nM/A260
Absorbance	35.0 ug/A260
Percent GC	57.9 %
Delta G	-29.4 kcal/mol
Delta H	-138.5 kcal/mol
Delta S	-359.0 eu
3. End Delta G	-5.4 kcal/mol
Delta G	25.0 degrees C
Probe concentration	0.6 pMol
Salt concentration	1000.0 mMol
Formamide concentration	0.0 %
3. End length	7 bases
Run length	4 bases
Palindrome length	8 bases
Hairpin loop stem length	3 bases

Structural Analysis Summary

Number of base runs	/ Palindromes	0 / 0
Number of hairpin loops	/ 2-oligo dimers	0 / 0
Number of dimers	/ 2-oligo bulges	0 / 0
Number of bulge loops	/ 2-oligo internals	0 / 0
Number of internal loops	/ 2-oligo	0 / 0

Analysis of "table 28 (S1MB primer 12S-L)" a 21-mer DNA Oligonucleotide(Sense)

5' GGC TAA CTA CAA TCA TCT GCT 3'

Oligonucleotide Analysis

	Analysis Parameters
Molecular weight	6445.2
ta thermodynamic	58.5 degrees C
% GC	50.9 degrees C
AT+GC Tm	66.9 degrees C
Absorbance	60.0 degrees C
Percent GC	5.1 nmol/A260
Delta G	32.6 ug/A260
Delta H	42.9 %
Delta S	-30.8 kcal/Mol
3' End Delta G	-153.4 kcal/Mol
	-403.9 eu
	-6.3 kcal/Mol.

Structural Analysis Summary

Number of base runs	/	Number of palindromes	/	0 / 0
Number of hairpin loops	/	Number of 2-oligo dimers	/	0 / 0
Number of dimers	/	Number of 2-oligo bulges	/	0 / 0
Number of bulge loops	/	Number of internal loops	/	0 / 0
Number of internal loops	/	Number of 2-oligo internals	/	0 / 0